ASTAXANTHIN BASED INDICATOR FOR LIGHT METALS IN *Haematococcus pluvialis*

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ABSTRACT

Pollution of metals has concerned as a crucial issue with regards to environmental, human health and industrial biotechnology. Today, various studies were highlighted heavy metals as inducer for production of astaxanthin in *Haematococcus pluvialis*. However, using light metals to induce the production of astaxanthin in *H. pluvialis* so far have not been highlighted. In this research, various light metals would be used to induce astaxanthin production in *H. pluvialis*. The astaxanthin produced in *H. pluvialis* is then enabling to indicate whether the light metals causing stress and toxicity towards the *H. pluvialis*. In this research, Al, Li and Mg with different concentrations were chosen to induce the astaxanthin production in *H. pluvialis*. In the other word, there was also focus on the effect of light metals with different pH toward the astaxanthin production in *H. pluvialis*. Al, Li & Mg with different pH and concentrations were exposed to *H. pluvialis* cells. In this case, the astaxanthin production was measured using spectrophotometric methods by exposure to light metals with several durations. After study the topic, the effect of light metals with different pH and concentrations toward astaxanthin production in *H. pluvialis* would indirectly shows that whether astaxanthin considered as a sensitive indicator for light metals. The astaxanthin production from *H. pluvialis* cells able to be measuring by detection of absorbance using light spectrophotometer at 482 nm. From the research, the Mg shows highest percentage of astaxanthin production if compared to Al and Li. This had indicated that there were different oxidative stresses conducted by different light metals. Besides that, pH 7 of Al solution also shows the highest percentage of astaxanthin production when compared to other pH values. This also indicated that different pH causes a different level of oxidative stress towards *H. pluvialis*. As a summary, astaxanthin was proven as a sensitive indicator for light metals with different pH and concentrations. It also has ability to differentiate the different level oxidative stresses causing by different type of light metals.

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LIST OF ABBREVIATION

%	Percentage
Al	Aluminum
BBM	Bold's Basal Medium
°C	Degree celsius
$C_{40}H_{52}O_4$	Astaxanthin
CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
g	Gram
g/mol	Grams per mole
H. pluvialis	*Haematococcus pluvialis*
HCl	Hydrochloric acid
kflux	Kiloflux
Li	Lithium
M	Molarity
Mg	Magnesium
mg/L	Milligrams per Litre
mL	Millilitre
nm	Nanometers
OD	Optical Density
OH	Hydroxyl group
ppm	Parts per million
ROS	Reactive Oxygen Species
rpm	Revolutions per Minute
UV	Ultraviolet
% (w/v)	Percentage weight over volume

CHAPTER 1

INTRODUCTION

Bio-indicators defined as "analytical device" comprising of a biological element which functions to analyze samples for the presence of a specific analyte (Xia, Zhang, Zhao, & Bunn, 2014). A bio-indicator has many kinds of applications in different fields such as clinical diagnostic, industrial application to monitor manufacturing processes and environmental application that help in detecting the toxicity and quality of the natural environment (Stavropoulos, Chantzis, Doukas, Papacharalampopoulos & Chryssolouris, 2013). However, existing bio-indicators require expensive resource in the manufacturing process to ensure the accuracy of analysis (Chen et al., 2012). Therefore, scientists often look for a replacement to the existing bio-indicators and they found that algae can be a good indicator as its reaction with some elements such as metals to produce a specific compound which is astaxanthin (Miazek, Iwanek, Remacle, Richel & Goffin, 2015).

Astaxanthin is a red pigmented member of the carotenoid family that exists in many organisms (Ambati et al., 2014). Astaxanthin is commonly formed in marine organisms such as salmon, lobster, and prawn. It was distinguished by the capacity to interaction with chemically reactive oxygen and free radicals (Fassett & Coombes, 2011). Except for marine organisms, other organisms such as human cannot synthesize astaxanthin naturally. The reason of accumulation of astaxanthin from their diet is to protect lipid cells from peroxidation (Fassett & Coombes, 2011).

H. pluvialis was reported as the main source of natural astaxanthin. There were articles pointed out that when *H. pluvialis* facing oxidative stress, it will accumulate astaxanthin to protect itself by quenching the reactive oxygen species (ROS) and free radicals brought by the source of oxidative stress. To date, heavy metals have been regularly studied about the toxicity towards the environment by exposure to the *H. pluvialis* to observe the accumulation of astaxanthin in the cells. But, currently fewer studies about toxicity of light metals as a source of oxidative stress to stimulate the production of astaxanthin in *H. pluvialis*.

Due to the low toxicity level, light metals were widely used in industrial products such as Al was popularly used as cans for storage and preservation of foods. Besides that, Li was also another light metals that using daily by humans due to its unique chemical properties in mechatronic products. These light metals released by industrial can cause serious respiratory disease to human and living organisms (Exley, Swarbrick, Gherardi, & Authier, 2009). Although Mg is an essential nutrients that required by living organisms, but large amounts of Mg accumulated in our living environment can also cause respiratory disease (Landon & Young, 1993). For the environmental sides, light metals industrial products that readily discarded by human beings after used were also highly affecting ecosystem. The wild living organisms might accidently ingested the light metals products and lead to deadness (Bearne, Dupuis, & Tarcy, 2017).

As a summary, the aim of this experiment is to investigate the effects of light metals such as Al, Li, and Mg with different concentrations towards the production of astaxanthin in microalgae *H. pluvialis*. This can indirectly find out whether astaxanthin is suitable to be used as bio-indicator for Al, Li and Mg compounds. Last but not least, light metals with different pH would also be exposed to the cells to determine the effect of pH towards the astaxanthin production in *H. pluvialis*.

CHAPTER 2

LITERATURE REVIEW

2.1 PROPERTIES OF ASTAXANTHIN

2.1.1 Structure of Astaxanthin

Astaxanthin belongs to the class of β-carotene family and has a long chain of 40 carbons (3,3'-dihydroxy-β, β '-carotene-4,4'-dione), which are known as 3,3'-dihydroxy-β, β'-carotene-4,4'-dione with a molecular formula of $C_{40}H_{52}O_4$ (Wu et al., 2015). Naturally, astaxanthin is widely distributed in plants, aquatic animals, algae, and yeast as an orange-red colored pigment (Wu et al., 2015). In the medical application, astaxanthin has a very strong anti-tumor and antioxidant capacity (Zhang & Wang, 2015); when biological peroxidation is inhibited, the process of mutagenesis of cancer cells is also inhibited. It can inhibit the oxidation of unsaturated fatty acids in blood, this reducing the deposition of blood vessel wall, inhibit atherosclerosis, prevention and treatment of neurological diseases, such as Parkinson's disease (Zhekisheva, Zarka, Khozin-Goldberg, Cohen, & Boussiba, 2005).

The astaxanthin molecular structure similar with many other carotenoids has a long conjugate double bond chain but also in the end of conjugated double bond chain and unsaturated ketone and hydroxyl, ketone and hydroxyl and constitute the α-hydroxy ketone (Butnariu, 2016). Astaxanthin contains two identical centres presented at the positions of 3 and 3. From this, 3 stereoisomers of astaxanthin can be form as shows in Figure 2.1 depending on the spatial orientation of the OH groups in chiral carbon (Higuera et al., 2006). These structures have a more active electronic effect, can attract free radicals or provide electrons, thereby eliminating free radicals (Liu et al., 2014).

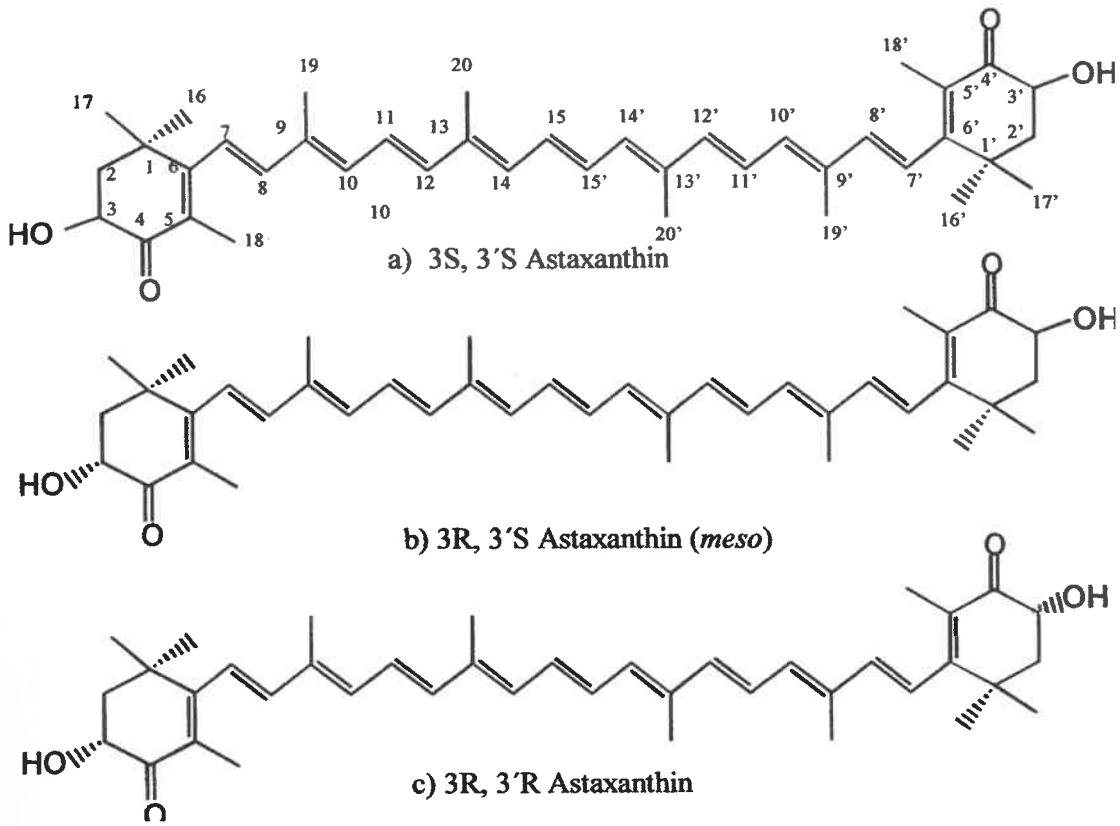

Figure 2.1: Stereoisomers of astaxanthin (Higuera-Ciapara et al., 2006).

Due to the structure of astaxanthin contains more -OH groups and C=O groups, greatly enhance the hydrophilicity of the molecule, making astaxanthin both lipophilic and hydrophilic, so that it can cross the cell membrane phospholipid bilayer structure and cause the role of antioxidant in both intracellular and extracellular (Liu et al., 2014).

2.1.2 Antioxidant activity of astaxanthin

Large numbers of studies shows that astaxanthin has a strong ability of quenching singlet oxygen, stronger than zeaxanthin, lutein, class beetle yellow, β - carotene 500 times more than vitamin E (Shah, Liang, Cheng & Daroch, 2016). It is deduced that the ability to quench the reactive oxygen species increases with the number of conjugated double bonds (Ambati, Moi, Ravi & Aswathanarayana, 2014). Another study found that the polar structure of the carotenoid hydroxyl group intergrated into the membrane bimolecular layer hindered its polyolefin chain and singlet oxygen reaction, on the other hand, the structure containing hydroxyl and ketone-based

astaxanthin had higher antioxidant activity than zeaxanthin-containing only hydroxyl groups (Ambati et al., 2014).

Polar ends of astaxanthin (Super Vitamin E) as bridge-like molecules across the cell membrane to increase stability and mechanical strength of the cell membrane (Higuera, Valenzuela & Goycoolea, 2006). According to Higuera et al., (2006), vitro experiments had been performed and proven that the powerful antioxidant activity of astaxanthin was cause by its stable structure which is able to reduce membrane permeability, limiting the peroxide promoters such as H_20_2 tertbutyl base hydrogen peroxide (t-ButOOH) and ascorbic acid into the cell, protecting cells from oxidative damage important molecules. Therefore, the astaxanthin accumulate in *H. pluvialis* can be concluded as a protection mechanisms when it facing oxidative stress. This is because the antioxidant activity of astaxanthin allowed reducing the singlet oxygen and eliminating the free radicals brought by the oxidative source (Miazek, Iwanek, Remacle, Richel & Goffin, 2015).

2.2 DERIVATION OF ASTAXANTHIN IN *H. Pluvialis*

2.2.1 Microalgae *H. pluvialis* as main source of natural astaxanthin

Astaxanthin is widely found in the ecosystem, especially in aquatic animals such as shrimp, crab, fish, and algae (Mezzomo & Ferreira, 2016). The microalgae *H. pluvialis* is the ideal organism used to obtain of natural astaxanthin as it has the highest content of astaxanthin organisms (Wu et al., 2015). Astaxanthin in *H. pluvialis* (1.5% to 3.0%) is seen to be the most concentrated natural astaxanthin existing in the world (Shah et al., 2016). Numerous studies shows that the rate and total production of raw accumulation astaxanthin in *H. plixialis* are higher than other green algae (Ambati et al., 2014). In addition to that, the structure of astaxanthin in *H. pluvialis* is mainly 3S-3'S-type, which is basically the same as the structure of astaxanthin in marine organisms such as salmon (Liu et al., 2014).